

APPENDIX A - Protocol



PROTOCOL For STUDY 24074-20

Test Substance: BEHR Antibacterial Paint, #3190
Study Title: ACUTE DERMAL IRRITATION in RABBITS
Guideline: OCSPP 870.2500
Test Facility: STILLMEADOW, Inc.
12852 Park One Drive
Sugar Land, TX 77478

Approved: Vincent A. Murphy 04 Nov 20
Vincent A. Murphy, PhD, DABT
Study Director, STILLMEADOW, Inc. Date

Approved: [Signature] 02 Nov 20
Management, STILLMEADOW, Inc. Date

Reviewed: Kristina Rodrigue 02 Nov 20
Kristina Rodrigue, RQAP-GLP
Quality Assurance Director, STILLMEADOW, Inc. Date

Sponsor: BEHR Paint Company
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Approved: John A. Gilbert 11/03/2020
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Chief R&D Officer Date

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A. GENERAL

1. Study Title: Acute Dermal Irritation in Rabbits
2. Purpose: To assess relative level of primary skin irritation produced when rabbits are exposed to test substance under semiocluded conditions.
3. Method Guidelines: This study will be conducted according to US OCSPP 870.2500.
4. Regulatory Compliance: This study will be conducted in compliance with Good Laboratory Practice (GLP) standards:
 1. EPA FIFRA 40 CFR 160
In the event of a regulatory inspection, Regulatory Inspectors will be provided with all study documentation requested. Sponsor will be notified of inspection of their study. All procedures in this protocol are in compliance with Animal Welfare Act Regulations. All methods can be found in STILLMEADOW, Inc. Standard Operating Procedures (SOP).
5. Quality Assurance: The Quality Assurance Unit (QAU) will review the protocol. Study information will be entered into the master schedule. In-progress inspection(s) will be performed to ensure integrity of the study. Any deviations from SOP, protocol or GLP standards will be reported to Study Director and Management. Raw data and report will be audited, and a statement prepared and signed which will specify dates inspections were made and findings reported to Management and Study Director.
6. Test Substance: BEHR Antibacterial Paint, #3190. Test substance identification should include name, lot/batch number and purity. Sponsor should also provide information regarding safety, storage conditions and disposal. Sponsor assumes responsibility for purity, stability, identity, synthesis methods and location of documentation.
7. Proposed Schedule: Testing should begin after test substance receipt, authorization to conduct study and study initiation.
Proposed Experimental Start & End: 10 Nov 20 - 24 Nov 20
In-life: at least 72 hours; maximum 14 days
8. Study Director: Vincent A. Murphy, PhD, DABT
9. Experimental Summary: Test substance will be applied to a single intact skin test site on each test rabbit and maintained in contact with skin for 4 hours. Test sites will then be washed scored for signs of skin irritation on Day 0 at appropriate times, and again at ~24, 48 and 72 hours after end of the exposure period (post patch removal); also every 2 to 4 days after until reversible irritation subsides, or maximum of 14 days. Primary Irritation Index (PII) will be determined from scores through 72 hours. Toxicity Category may also be assigned.
10. Protocol Amendments: Any protocol alteration will be justified, approved by Study Director and recorded in writing.
11. Sponsor Audits: Sponsor may send an authorized Representative to inspect test system and/or data on STILLMEADOW, Inc. premises during normal working hours.

B. EXPERIMENTAL DESIGN

1. Animals
 - a. Species/Strain/Source: Albino rabbit / New Zealand White / Robinson Services Inc; Mocksville, NC (or other suitable source)

- B. 1. b. Species Justification: The rabbit is conventionally used in primary dermal irritation studies to provide information on which human hazard can be judged, and is preferred by regulatory agencies.
- c. Quantity & Sex: 3 rabbits; male &/or female (nulliparous & non-pregnant)
- d. Age/Weight at Dosing: 12 week - 6 month / 2 - 4 kg
- e. Identification: Ear tag, tattoo or marker
- f. Acclimation & Health Status: Animals will be acclimated for at least 5 days prior to dosing. Normal weight gain, appearance and behavior will be factors used to select healthy naive animals for testing.
2. Animal Husbandry
- a. No./Cage & Cage Type: Individually housed in stainless steel, suspended, wire bottom cage
- b. Enrichment: Provided to each animal during study
- c. Food: Measured amount of Rowe Nutrition RSI Breeder Hi-Fiber Diet or equivalent; analyzed by manufacturer for nutritional content
- d. Water: Tap water, available ad libitum (automatic system); municipal water supply analyzed by TCEQ Water Utilities Division
- e. Contaminants: There are no known contaminants in feed or water available to laboratory animals that would be expected to interfere with this study.
- f. Environment: Target temperature: 20° ± 3°C Target relative humidity: 30 - 70%
12-hr light/12-hr dark cycle (regulated automatically)
Room ventilation: at least 10 air changes per hour
3. Test Substance Administration
- a. Animal Preparation: On the day prior to treatment, dorsal area of the trunk of each animal will be clipped free of hair to expose ~8 x 8 cm area. Animals with exposure areas free from pre-existing skin irritation or defects will be selected for testing. A single intact exposure site will be selected as test site.
- b. Reason for Route of Administration: Dermal contact is a potential route of human exposure.
- c. Test Substance Application: On Day 0, 0.5 mL liquid or 0.5 g solid or semi-solid test substance will be introduced under ~2.5 x 2.5 cm surgical gauze patch to a single ~6 cm² test site on each of three animals. Solid test substances will be moistened with DI water to form thick paste prior to application and may require a larger gauze patch to cover all test substance. If water cannot be used as moistener, acceptable alternatives are saline, corn/mineral oil, glycerol, ethanol and water, aqueous CMC and gum arabic. In some cases, test substance may be applied to the gauze patch and the patch placed on the skin. The entire trunk will be covered with semiocclusive dressing for a 4 hour period.
- d. Control Site: Contralateral area of untreated skin will serve as control against which reactions of treated site are evaluated.
- e. Test Substance Removal: After the 4-hour exposure period, patches and wrappings will be removed and test substance removed as thoroughly as possible using water and/or appropriate non-irritating solvent (e.g., mineral oil); dry-wiping may alternatively be done. Control site will be treated in a similar manner.

- B. 3. f. Alternate Stepwise Exposure of Animals: If suspected that test substance might produce severe irritation/corrosion, a single rabbit may be initially used, with three test patches applied concurrently or sequentially to the animal. The first patch is removed after 3 minutes; if no serious skin reaction is observed, the second patch is removed after 1 hour. If observations indicate exposure can continue humanely, the third patch is removed after 4 hours and responses graded. If corrosive effect is observed after exposure of up to 4 hours, further animal testing is not required. If no corrosive effect is observed after 4-hour exposure, test is completed using additional animals, each with one patch only, for exposure of 4 hours.
4. Observations
- a. Dermal Irritation: Animals will be scored for erythema, edema and other signs of dermal defects 30 - 60 minutes after removal of patches, and at ~24, 48 and 72 hours after end of the exposure (final patch removal). If irritation persists through 72 hours, scoring will be done every 2 to 4 days after until all reversible irritation subsides (maximum of 14 days). Draize technique Scoring Scale for signs of dermal irritation is presented in Legend A. At the first observation, if any, of excessive irritation causing/expected to cause pain/distress, a sufficient concentration of suitable analgesic will be administered by subcutaneous or IM injection. Animal will be monitored and analgesic readministered at appropriate frequency until irritation lessens.
- b. Other Observations: Observations of toxic effects, if any, may be recorded. Animals that have a damaged skin producing undue stress/discomfort will be humanely sacrificed after consulting Sponsor; each study animal will be weighed and euthanized following its final observation.
5. Evaluation of Results: For each animal, all erythema and edema scores through 72 hours will be added and the sum divided by those number of scoring periods to obtain an individual irritation score. PII will be determined by calculating mean of irritation scores for all tested animals and used to give test substance a descriptive rating according to Classifications in Legend A. Toxicity Category may be assigned according to criteria as follows.
- | <u>Toxicity Category</u> | <u>Criteria (per Proposed Rule, FR Vol. 49, No. 188)</u> |
|--------------------------|--|
| I | Corrosive (anytime) |
| II | Severe irritation at 72 hours |
| III | Moderate irritation at 72 hours |
| IV | Non-irritating to slight irritation at 72 hours |
6. Test Substance Accountability: A comprehensive inventory of test substance received and used will be kept. Test substance container(s) will be weighed when received at this facility, and all test substance use recorded. Test substance and test substance dosing solutions will be stored in original containers or equivalent, or in capped glass containers.
7. Unused Test Substance Disposal: Unused test substance will be disposed of at Sponsor's expense after study termination.
8. Safety Precautions: General safety precautions required by laboratory SOP will be followed. Sponsor will supply basic toxicity data on test substance to be used; however, since toxicity of test substances is often not well characterized, this laboratory will be conservative in setting safety procedures. Sponsor or Representative shall be notified of any exposure requiring physician's exam or care.

C. DATA MANAGEMENT

1. Records: The following records will be maintained at STILLMEADOW, Inc. during the study, and archived upon study termination:
 - a. Protocol & protocol amendments (if any)
 - b. Final report & amendments (if any)
 - c. Study correspondence
 - d. Animal receipt/acclimation data
 - e. Test substance receipt, identification supplied by Sponsor, preparation, administration, disposition
 - f. Test animal information: number, sex, source, strain
 - g. All observations & scores for skin irritation for all time periods
 - h. Other toxic effects if observed
 - i. Other pertinent data
2. Data Storage: All raw data, originals of protocol, final report, any amendment(s) and a test substance sample will be archived at STILLMEADOW, Inc. for 15 years.
3. Data Reporting: Final report will include following data as described in GLP standards:
 - a. Statement from QAU
 - b. GLP Compliance Statement & signature of Study Director
 - c. Names of scientific personnel involved in study
 - d. Dates of study initiation & termination
 - e. Identification, label information, description, preparation, storage of test substance
 - f. All pertinent animal data & husbandry, dosing information, observation methods
 - g. Description of test procedures
 - h. Identification of moisteners, if any, used
 - i. Individual scores for erythema & edema at all observation periods; tabulation of dermal effects data
 - j. PII score & descriptive rating for test substance; Toxicity category (if assigned)
 - k. Other toxic effects if observed
 - l. Copy of this protocol; deviations (if any) & impact on study
4. Report Generation: A final report will be generated after termination of in-life portion of the study; a draft report may first be issued for Sponsor approval.

LEGEND A

Primary Dermal Irritation Scoring Scale (Draize Technique*)

<u>Erythema Formation</u>	<u>Score</u>	<u>Edema Formation</u>	<u>Score</u>
None	0	None	0
Very slight (barely perceptible)	1	Very slight (barely perceptible)	1
Well-defined	2	Slight (edges well defined)	2
Moderate	3	Moderate (raised ~1 mm)	3
Severe (beet redness to eschar preventing scoring of erythema)	4	Severe (raised >1 mm & beyond test area)	4
Maximum Possible	4	Maximum Possible	4

Other observations may be noted, for example: desquamation, necrosis, etc.

* - Draize, John H, Woodard, Geoffrey, & Calvery, HO, "Methods for the Study of Irritation & Toxicity of Substances Applied Topically to the Skin & Mucous Membranes." J. Pharm. & Ther. 82, 377 (1944).

Classification of Test Substance

<u>Descriptive Rating</u>	<u>Primary Irritation Index</u>	<u>Descriptive Rating</u>	<u>Primary Irritation Index</u>
Non-irritating	0.0	Moderately Irritating	2.0 - 5.0
Slightly Irritating	0.1 - 1.9	Severely Irritating	5.1 - 8.0